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13. ABSTRACT *(Maximum 200 words)*

Acetazolamide (AZ) can be taken at sea level (SL) to prevent acute mountain sickness during subsequent altitude (ALT) exposure. AZ causes metabolic acidosis at SL and ALT, and increases arterial oxygen saturation (SaO2) at ALT. HYPOTHESIS: AZ will impair muscle endurance at SL but not simulated ALT (4300 m, < 3 h). METHODS: Six subjects (20 ± 1 yr; X±SE) performed exhaustive constant work rate 1-leg knee extension exercise (25 ± 2 watts) once per week for 4 weeks; twice at SL and twice at ALT. Each week, subjects took either AZ (250 mg) or placebo orally in double blind fashion (t.i.d.) for two days. On day 2, all exercise bouts began ~2.5 h after the last dose of AZ or placebo. RESULTS: AZ caused similar acidosis (pH) in all subjects at SL (placebo: 7.43 ± 0.01 vs. AZ: 7.34 ± 0.01, $P<0.05$) and ALT (placebo: 7.48 ± 0.03 vs. AZ: 7.37 ± 0.01, $P<0.05$). However, endurance performance was impaired with AZ only at SL (placebo: 48 ± 4 min vs. AZ: 36 ± 5 min, $P<0.05$) and not ALT (placebo:17 ± 2 min vs. AZ: 20 ± 3 min, ns). CONCLUSION: Lack of endurance performance impairment of AZ compared to placebo at ALT was likely due to offsetting secondary effects resulting from the acidosis e.g., ventilatory-induced increase in SaO2 for AZ vs placebo (89 ± 1% vs 86 ± 1%, $P<0.05$) that resulted in an increased O2 pressure gradient from capillary to exercising muscle.

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Effect of acetazolamide on leg endurance exercise at sea level and simulated altitude

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ABSTRACT

Acetazolamide can be taken at sea level to prevent acute mountain sickness during subsequent altitude exposure. Acetazolamide causes metabolic acidosis at sea level and altitude, and increases Sa_{O_2} (arterial oxygen saturation) at altitude. The aim of the present study was to determine whether acetazolamide impairs muscle endurance at sea level but not simulated altitude (4300 m for < 3 h). Six subjects (20 ± 1 years of age; mean ± S.E.M.) performed exhaustive constant work rate one-leg knee-extension exercise (25 ± 2 W) once a week for 4 weeks, twice at sea level and twice at altitude. Each week, subjects took either acetazolamide (250 mg) or placebo orally in a double-blind fashion (three times a day) for 2 days. On day 2, all exercise bouts began approx. 2.5 h after the last dose of acetazolamide or placebo. Acetazolamide caused similar acidosis (pH) in all subjects at sea level (7.43 ± 0.01 with placebo compared with 7.34 ± 0.01 with acetazolamide; $P < 0.05$) and altitude (7.48 ± 0.03 with placebo compared with 7.37 ± 0.01 with acetazolamide; $P < 0.05$). However, endurance performance was impaired with acetazolamide only at sea level (48 ± 4 min with placebo compared with 36 ± 5 min with acetazolamide; $P < 0.05$), but not altitude (17 ± 2 min with placebo compared with 20 ± 3 min with acetazolamide; $P =$ not significant). In conclusion, lack of impairment of endurance performance by acetazolamide compared with placebo at altitude was probably due to off-setting secondary effects resulting from acidosis, e.g. ventilatory induced increase in Sa_{O_2} for acetazolamide compared with placebo (89 ± 1 compared with 86 ± 1 % respectively; $P < 0.05$), which resulted in an increased oxygen pressure gradient from capillary to exercising muscle.

INTRODUCTION

AMS (acute mountain sickness) is a symptom complex that includes headache, nausea, dizziness, tiredness, weakness and insomnia, and is most common when low-altitude residents ascend rapidly to altitudes exceeding 3000 m [1]. Acetazolamide has been used for decades to prevent AMS [2]. It inhibits carbonic anhydrase and causes increased loss of bicarbonate, water, sodium and potassium in the urine, a reduced concentration of bicarbonate in extracellular fluid, metabolic acidosis and increased ventilation [2–4]. When taken prophylactically as indicated (i.e. 24–48 h prior to ascent), acetazolamide prevents AMS in 30–50 % of individuals and reduces

Key words: acute mountain sickness, acetazolamide, fatigue, hypoxia, isolated muscle exercise, metabolic acidosis, ventilation.

Abbreviations: AMS, acute mountain sickness; AMS-c, AMS cerebral symptom; AMS-r, AMS respiratory symptom; DKE, dynamic knee extension; ESQ, environmental symptoms questionnaire; LED, light-emitting diode; MVC, maximal voluntary contraction; Pa_{O_2}, partial pressure of arterial oxygen; P_{O_2}, partial pressure of oxygen; Sa_{O_2}, arterial oxygen saturation; USARIEM, United States Army Research Institute of Environmental Medicine; $\dot{V}_{CO_2}$, carbon dioxide output; $\dot{V}_E$, minute ventilation; $\dot{V}_{O_2}$, oxygen uptake; $\dot{V}_E/\dot{V}_{CO_2}$, ventilatory equivalent for carbon dioxide; $\dot{V}_E/\dot{V}_{O_2}$, ventilatory equivalent for oxygen.

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symptoms in most others [5]. It is currently the only drug approved by the Food and Drug Administration for this purpose [2].

Although acetazolamide has been demonstrated convincingly to prevent or reduce AMS symptoms in most individuals, collective findings from previous studies indicate that acetazolamide impairs, does not affect or improves maximal and submaximal exercise performance at sea level or altitude [4,6–12]. The inconsistent findings may be related to a difference in the relative balance of opposing physiological responses associated with acetazolamide treatment. On the one hand, metabolic acidosis itself impairs the ability to buffer metabolic acids in active muscle and thereby tends to hinder exercise performance at sea level and altitude [13–15]. On the other hand, the opposing secondary responses resulting from the acidosis, an elevation in $\dot{V}_E$ (minute ventilation) that may enhance oxygen delivery to active muscle despite potentially impaired oxygen loading at the lung (i.e. the Bohr effect), tend to improve exercise performance, but would be useful only at altitude where P_{O_2} (partial pressure of oxygen) and S_{aO_2} (arterial oxygen saturation) are reduced [6,11].

Whether this hypothesis is true has been difficult to determine because: (i) exercise performance at altitude using conventional ergometry (e.g. whole-body treadmill or bicycle ergometry) may have been restricted by central circulatory and pulmonary diffusion limitations [16]; (ii) the use of different individuals or exercise intensities and power outputs at altitude compared with sea level make performance comparisons difficult to interpret [12]; and (iii) AMS may have been present in some studies during drug and performance assessments [6].

The present study was designed to minimize such confounding factors and determine the effect of acetazolamide on muscle endurance exercise performance at sea level and simulated altitude. This was accomplished with the use of an exercise model with low test–retest variability [17] and no central circulatory or pulmonary diffusion limitations [18]. Endurance times of the same subjects performing isolated muscle exercise at identical power outputs under all experimental conditions were compared in the absence of AMS. The overall study hypothesis was that acetazolamide would impair exercise performance at sea level but not simulated altitude.

MATERIALS AND METHODS

Subjects

Five men and one woman (20 ± 1 years, 78 ± 2 kg and 173 ± 2 cm; values are means $\pm$ S.E.M.) voluntarily provided verbal and written consent to participate after being fully informed of the nature of the study in accordance with Declaration of Helsinki, the Human Research Review Committees of USARIEM (United States Army Research Institute of Environmental Medicine) and The Office of the Surgeon General. The investigators adhered to policies of applicable Federal Law CFR 46 for the protection of the subjects. All subjects were born at altitudes < 1500 m and resided near sea level (< 100 m) for at least 6 months before the study started.

Overall experimental timetable and location

A 3-week-long preliminary testing phase was followed by a 4-week-long definitive testing phase. The presentation of the definitive exercise testing bouts (i.e. sea level + placebo, sea level + acetazolamide, simulated altitude + placebo and simulated altitude + acetazolamide) was assigned randomly for each subject. Both the subjects and the investigators directly involved were blinded to drug treatment status until the entire study was completed. Each subject performed only one exercise bout each week during the definitive testing phase. With the exception that the subjects were not allowed to run or perform leg-weight-training exercise at least 1 day prior to all exercise bouts in the preliminary and definitive testing phases, individual physical activity levels were maintained throughout the 7-week study. Preliminary and definitive phase testing took place in the Altitude Chamber Facility, USARIEM, Natick, MA, U.S.A. Temperature and relative humidity were maintained at 21 ± 1 °C and 45 ± 2 % respectively.

Preliminary testing phase

The subjects were familiarized with the altitude chamber and practised the test procedures that would be used during the definitive testing phase. In addition, baseline $\dot{V}_{O_2}$ (oxygen uptake) peak values were determined for graded bicycle ergometer and DKE (dynamic knee extension).

Definitive testing phase

Placebo or acetazolamide treatment and several experimental procedures took place over two consecutive days in each of the four successive weeks. Any medication, caffeine and carbonated beverages were strictly prohibited from the beginning of day 1 until after the DKE exercise bout on day 2. For each subject, the week-to-week experimental schedule and data collection procedures were identical, except for presentation of sea level/simulated altitude days and placebo/acetazolamide treatments.

Day 1 Subjects reported to the Altitude Chamber Facility at 07.00 and 15.30 hours, i.e. 27 h and 18.5 h prior to the exercise bout at 10.00 hours on day 2 ($t = 0$ h). At both 07.00 and 15.30 hours, subjects swallowed a placebo or acetazolamide capsule in the presence of an investigator. During the second visit to the Altitude Chamber Facility, subjects were provided with a third

capsule and instructed to ingest it either just before sleep or no later than 22.00 hours on day 1.

Day 2 Subjects reported to the Altitude Chamber Facility at 07.00 hours after an overnight fast that began at 20.00 hours on day 1. After body weight was measured, subjects were provided with a standardized light snack [i.e. 420 kcal (1 kcal ≡ 4.184 kJ) of which 65 % was carbohydrate] consisting of a commercially available energy bar and peanut butter crackers. At 07.30 hours, subjects took the fourth and last placebo or acetazolamide capsule of the week and were required to remain near the Altitude Chamber Facility. At 09.30 hours, an arterialized capillary blood sample was taken, and then subjects were prepared (i.e. electrodes placed, secured to knee extension device etc.) for DKE testing. An ESQ (environmental symptoms questionnaire [19]) was completed just prior to the start of DKE exercise at 10.00 hours.

The times of the above events remained identical for bouts at both sea level and simulated altitude with the exception that, during the simulated altitude testing bouts, the chamber (containing the volunteer and investigative staff) was decompressed starting at 08.05 hours at a rate of 45 mmHg/min to a pressure of 446 mmHg (equivalent to 4300 m altitude). Decompression to 4300 m took approx. 10 min.

Placebo/acetazolamide treatment

Acetazolamide and an identically appearing placebo (lactose) capsule were prepared by a local pharmacy that had no other relationship with the study. The initiation of treatment (i.e. day before ascent), the 250 mg dose and the administration frequency are consistent with current recommendations for acetazolamide treatment prior to altitude exposure [2,5].

Test procedures and measurements

$\dot{V}O_2$peak during conventional bicycle ergometer exercise

$\dot{V}O_2$peak was determined during continuous graded bicycle exercise on an electrically braked ergometer once at sea level during the preliminary testing phase. A pedal rate of 65–70 rev./min was used. Subjects warmed-up for 3 min at 100 W with the work rate increased by 30 W every 2 min thereafter.

$\dot{V}O_2$peak was defined as the point at which $\dot{V}O_2$ began to plateau with increased work rate or at the point where the volunteer could no longer maintain the work rate despite strong encouragement.

$\dot{V}O_2$peak during DKE exercise

$\dot{V}O_2$peak was determined during graded DKE to peak exercise once at sea level during the preliminary testing phase. Graded DKE consisted of 4-min stages of one-leg DKE exercise of graded intensity separated by 4 min of complete rest. Increments of work rate applied to each stage to exhaustion were individually determined for each subject.

$\dot{V}O_2$peak was defined as the highest value just prior to a deviation from a linear relationship between a change in $\dot{V}O_2$ and a change in work rate [18]. A total of 4–7 exercise stages were used for each subject. Data collection procedures were identical to those described below.

Arterialized capillary blood sample

An arterialized blood sample of 100–200 μl from the fingertip was obtained during day 2 of each week during the four definitive exercise testing days ($t = -0.5$ h). During the two simulated altitude testing days, the blood samples were obtained after the subjects had been at simulated altitude for approx. 1.5 h. Arterialization was achieved by warming the hand in 38 °C water for 5 min to enhance regional blood flow. The sample was analysed on a blood gas analyser (ABL555; Radiometer) for pH, P_{O_2} (partial pressure of oxygen) and bicarbonate concentration. All staff members involved in the exercise testing were blinded to the blood analyses results until the end of the entire study.

Assessment of symptoms

The ESQ is a self-reported 68-item inventory typically used to document symptoms induced by altitude and other stressful environments. A total of the items and a weighted average of scores from 'cerebral' symptom items [AMS-c (AMS cerebral symptom)] and from 'respiratory' symptom items [AMS-r (AMS respiratory symptom)] were calculated [19]. To indicate sickness, AMS-c must be > 0.70 and AMS-r must be > 0.60.

Knee extension exercise

The specially designed device for performing one-leg (right leg) DKE exercise interspersed with maximal static one-leg knee extension contractions has been described in detail previously [18]. Briefly, it consisted of a platform on which the subject sat, an attached minimal-friction weight-pulley system with an ankle harness, transducers for measurement of force (sensitivity 1.5 mV/kg; model SSM-250; Interface) and ankle displacement (model PT101-0100-111-1110; Celesco Transducer Products) during DKE and separate force transducers for measurement of force of static knee extension MVCs (maximal voluntary contractions). In order to control work rate precisely, two vertical columns of 14 LEDs (light-emitting diodes) were placed in front of the subject. The right LED column was wired in series to the position transducer such that the number of LEDs lit was proportional to ankle displacement during knee extension. The left LED column was connected to a synthesizer/function generator that automatically and sequentially lit from one (at the 90° knee angle starting position) to 14 (corresponding to ankle displacement on reaching 160° of knee extension) to one (return to 90° starting position) at a pre-determined knee extension rate of 1 Hz. To maintain correct distance and rate of DKE, the subject continuously matched the column

of LEDs controlled by leg movement with that controlled by the synthesizer/function generator. The LED units simplified subject and investigator monitoring of adherence to the required work rate. Because the knee extension movement encompassed 70° and there were 13 intervals between LEDs, the maximum allowable difference between the desired and actual knee extension angle was 5.38°.

Muscle exhaustion was defined as a mismatch of only one LED between the right and left LED columns for three consecutive knee extensions, despite strong verbal encouragement. This effectively meant that exhaustion was associated with an inability to complete the last 5° of knee extension contraction, from 155° to 160°, at the required contraction rate. Voltages proportional to force and ankle displacement were recorded continuously. Work rate (in W) was determined by multiplying mean force developed per contraction, distance of ankle movement during knee extension from 90° to 160° and rate of knee extension (1 Hz).

To measure the decline in force-generating capacity, the exercise device allowed performance of MVCs of the knee extensor muscles during brief ($\leqslant 5$ s) pauses in DKE. This procedure involved rapid disconnection of the ankle harness from the weight-pulley system, connection to a force transducer dedicated to measurement of MVC force, actual measurement of MVC force and reconnection to the weight-pulley system.

Determination of MVC force During the preliminary and definitive testing phases, the subjects performed three or more pre-exercise knee extensor MVCs with the right leg prior to DKE. The subject was instructed to provide maximal effort during each MVC. At least 1 min of rest followed each MVC. MVC force of the leg was then measured immediately prior to and at the end of every 2 min during and immediately following DKE. A knee angle of 90° was used for all MVCs. To minimize duration variability among MVCs, each MVC triggered an audible sound that lasted exactly 2.5 s. The subject was instructed to stop contracting immediately on cessation of the sound.

Submaximal constant work rate knee extension exercise For each subject, one-leg DKE at a frequency of 1 Hz was performed to exhaustion at the same individually determined constant work rate during all definitive exercise bouts at sea level and simulated altitude. To determine the correct work rate for each subject required 4–6 DKE exercise sessions during the preliminary phase at sea level without acetazolamide treatment [18]. For the first preliminary DKE session, a light work rate was used that allowed the subjects to exercise for more than 1 h. With each subsequent session, the work rate was increased until endurance time to exhaustion was 40–60 min. Once an appropriate work rate was determined for each of the subjects, it was used for all four definitive testing phase sessions. For all tests, subjects were blinded to elapsed exercise time.

The methods and procedures of the DKE exercise model as used by our laboratory have been described in detail previously [18,20]. The test–retest coefficients of variation for pre-exercise MVC, the point of exhaustion and time to exhaustion are 4.9%, 5.6% and 7.5% respectively [17].

Heart rate

Heart rate was determined by three-lead ECG (Cardiovit AT-6; Schiller) prior to and every 2 min during the $\dot{V}O_2$peak tests (DKE and bicycle ergometry) and all four DKE bouts during the definitive phase.

Respiratory gas exchange

Exercise respiratory data were collected continuously until exhaustion using a metabolic cart (model 2900; Sensormedics) during the $\dot{V}O_2$peak tests (DKE and bicycle ergometry) and all four DKE bouts during the definitive phase. The cart was calibrated with medical grade calibration gases prior to each test.

Ratings of perceived exertion

Ratings of perceived exertion localized to the active muscles were obtained before and every 2 min during DKE exercise (15 s prior to each MVC) using the Borg 6–20 scale [21]. RPE data were collected during the $\dot{V}O_2$peak tests (DKE and bicycle ergometer) and during all four DKE bouts during the definitive phase.

Sa_{O_2}

Sa_{O_2} was monitored continuously via non-invasive finger pulse oximetry (model N-200; Nellcor) during all four DKE bouts during the definitive phase.

Statistical analyses

Two-way ANOVA (acetazolamide treatment compared with placebo, and sea level compared with simulated altitude) with repeated measures on both factors was used for data analyses. Neuman–Keuls post-hoc test was used to evaluate significant main effects when they were detected. Except where indicated, values are means $\pm$ S.D., or individual values. For all statistical analyses, a P value of $\leqslant 0.05$ was considered statistically significant.

RESULTS

$\dot{V}O_2$peak during conventional cycle ergometry

$\dot{V}O_2$peak was 3038 ± 630 ml/min (range, 2325–4050 ml/min) or 39 ± 7 ml $\cdot$ min^{-1} $\cdot$ kg^{-1} of body weight (range, 30–48 ml $\cdot$ min^{-1} $\cdot$ kg^{-1} of body weight) at a work rate of 230 ± 32 W. At exhaustion, heart rate was 180 ± 10 beats/min and rating of perceived exertion was 18 ± 2.

Table 1 Resting values

Values are means ± S.D. *$P < 0.05$ compared with the placebo in the same environment. †$P < 0.05$ compared with the same treatment at sea level ; Main effect indicates pooled difference for location (simulated altitude compared with sea level; $P < 0.05$).

	Sea level		Simulated altitude	
Measurement	Placebo	Acetazolamide	Placebo	Acetazolamide
Body weight (kg)	77.3 ± 5	75.8 ± 5	77.3 ± 5	76.1 ± 5
pH	7.427 ± 0.022	7.339 ± 0.020*	7.478 ± 0.071	7.369 ± 0.027*
Bicarbonate (mmol/l)	28.4 ± 2	19.7 ± 2*	27.7 ± 5	19.0 ± 2*
P_{O_2} (mmHg)	91.8 ± 11	92.0 ± 11	41.6 ± 5†	46.2 ± 2*†
ESQ (total score)	6 ± 2	6 ± 2	12 ± 7	11 ± 5
Main effect for location				
AMS-c (weighted score)	0.00 ± 0.0	0.00 ± 0.0	0.13 ± 0.12	0.20 ± 0.25
AMS-r (weighted score)	0.05 ± 0.05	0.06 ± 0.07	0.12 ± 0.10	0.13 ± 0.10
Heart rate (beats/min)	66 ± 7	64 ± 10	71 ± 7	76 ± 10†
Sa_{O_2} (%)	99 ± 1	99 ± 1	75 ± 5†	82 ± 5*†
Main effect for location				

$\dot{V}_{O_2}$peak during DKE

$\dot{V}_{O_2}$peak was 975 ± 223 ml/min (range, 689–1273 ml/min) at a workload of 34 ± 5 W. At exhaustion, heart rate was 127 ± 17 beats/min and rating of perceived exertion was 15 ± 2. DKE $\dot{V}_{O_2}$peak was 32 ± 5 % of conventional cycle ergometry $\dot{V}_{O_2}$peak.

Body weight and arterialized blood values

Table 1 shows the values for fasting body weight and resting blood pH, bicarbonate concentration, P_{O_2}, ESQ score, heart rate and Sa_{O_2}. Fasting body weight tended ($P > 0.05$) to be lower during acetazolamide treatment compared with placebo treatment in each environment. Body weight was nearly identical between sea level and simulated altitude for the same treatment. These findings imply that there was a mild diuretic response to acetazolamide and a similar overall body hydration prior to exercise in both environments.

In each environment, acetazolamide treatment significantly ($P < 0.05$) lowered arterialized blood pH and bicarbonate concentration. Acetazolamide treatment was therefore successful in causing an expected and similar metabolic acidosis just prior to DKE exercise in each environment. Pa_{O_2} (partial pressure of arterial oxygen) was also increased significantly ($P < 0.05$) during acetazolamide treatment at simulated altitude.

The total score of the ESQ during acetazolamide treatment was nearly identical with the total score during placebo treatment in each environment. However, the total score at simulated altitude was greater than the total score at sea level (main effect for location; $P < 0.05$). The two weighted sickness scores, AMS-c and AMS-r, were affected little by acetazolamide treatment in either environment. The weighted sickness scores also did not increase at simulated altitude compared with sea level. Thus, during either placebo or acetazolamide treatment and testing at simulated altitude, the subjects did not suffer from AMS. Collectively, the ESQ data indicate that acetazolamide treatment did not induce a change in well-being during testing in either environment compared with placebo.

Resting heart rate was not affected by acetazolamide treatment in either environment, but did increase significantly ($P < 0.05$) from sea level to simulated altitude during acetazolamide treatment. For both the placebo and acetazolamide treatments, resting Sa_{O_2} was significantly ($P < 0.05$) lower at simulated altitude compared with sea level. There was no difference in Sa_{O_2} between treatments at sea level, but at simulated altitude Sa_{O_2} was significantly higher ($P < 0.05$) during acetazolamide treatment than during placebo.

MVC force and endurance time

MVC force prior to and at exhaustion from DKE exercise and endurance times are shown in Table 2. Neither acetazolamide treatment nor environment altered initial MVC force or MVC force at exhaustion [whether expressed as absolute force values (i.e. N) or as a percentage of initial MVC force]. Approx. 41 % of MVC force was lost from the initiation of exercise to the point of exhaustion regardless of treatment or environment.

Each subject exercised to exhaustion at the identical submaximal work rate (25 ± 2 W) during placebo and acetazolamide treatments at sea level and simulated altitude. The submaximal work rate during DKE was 72 ± 5 % of DKE peak work rate and required a $\dot{V}_{O_2}$ that was 25 ± 2 % of cycle ergometry $\dot{V}_{O_2}$peak and 79 ± 7 % of DKE $\dot{V}_{O_2}$peak. Endurance time to exhaustion was reduced significantly ($P < 0.01$) at simulated altitude compared with sea level by 63 ± 17 % during placebo treatment and by 43 ± 22 % during acetazolamide treatment. Endurance time to exhaustion at sea level also was

Table 2 MVC force and endurance time values during knee extension exercise

Values are means ± S.D. *$P < 0.05$ compared with the placebo in the same environment. †$P < 0.05$ compared with the same treatment at sea level. Main effect indicates pooled difference for location (simulated altitude compared with sea level; $P < 0.05$).

	Sea level		Simulated altitude	
Measurement	Placebo	Acetazolamide	Placebo	Acetazolamide
Initial MVC force (N)	672 ± 196	632 ± 181	623 ± 142	635 ± 174
MVC force at exhaustion (N)	386 ± 125	393 ± 164	360 ± 115	376 ± 159
MVC force at exhaustion (% of initial MVC)	58 ± 7	62 ± 12	58 ± 10	58 ± 15
Endurance (min)	48.3 ± 10	35.7 ± 12*	17.0 ± 5†	19.9 ± 7†
Main effect for location				

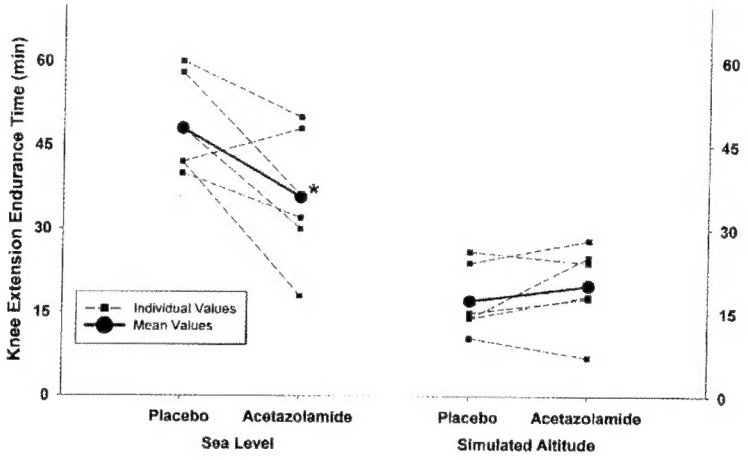

Figure 1 Individual and mean endurance times during knee extension exercise at sea level and simulated altitude with placebo and acetazolamide treatment

At sea level, endurance time declined for five subjects (range, −8 min to −24 min) and improved for one (+6 min) during acetazolamide treatment (overall mean change, −12 ± 4 min; *$P < 0.02$). In complete contrast, endurance times at simulated altitude declined for only two subjects (−2 min and −3 min), but improved for four (range, +3 min to +11 min) during acetazolamide treatment (overall mean change, +3 ± 2 min; P value was not significant).

reduced significantly ($P < 0.02$) during acetazolamide treatment by 26 ± 25% compared with placebo treatment. However, at simulated altitude for the same subjects, endurance time was similar for both treatments. Individual endurance times for sea level and simulated altitude during placebo and acetazolamide treatments are shown in Figure 1.

Steady-state exercise measurements

Because of the large changes in the intra-subject endurance times resulting from placebo at sea level (range, 40–60 min) to placebo at simulated altitude (range, 10–26 min) and from acetazolamide treatment at sea level (range, 18–50 min) to acetazolamide treatment at simulated altitude (range, 8–28 min), a representative steady-state exercise comparison (i.e. 50% of each subject's endurance time) among environments and treatments is shown in Table 3.

Exercise heart rate tended ($P > 0.05$) to be higher at simulated altitude than at sea level. Exercise heart rate was not affected by acetazolamide treatment in either environment. Rating of perceived exertion did not differ throughout each of the four definitive exercise testing sessions, although it tended ($P > 0.05$) to be higher during acetazolamide compared with placebo treatment at simulated altitude. In all testing sessions, leg exercise was perceived to be 'hard' to 'very hard'. Exercise $\dot{V}_E$ was significantly ($P < 0.05$) higher at simulated altitude than at sea level. At simulated altitude, but not at sea level, ventilation was significantly ($P < 0.05$) higher during acetazolamide treatment compared with placebo treatment. $\dot{V}_{O_2}$ and carbon dioxide production during exercise were not significantly affected by either acetazolamide or placebo treatment. Therefore $\dot{V}_E/\dot{V}_{O_2}$ (ventilatory equivalent for oxygen) and $\dot{V}_E/\dot{V}_{CO_2}$ (ventilatory equivalent for carbon dioxide; where $\dot{V}_{CO_2}$ is carbon dioxide output) were also significantly ($P < 0.05$) higher at simulated altitude compared with sea level. Sa_{O_2} was reduced from sea level to simulated altitude for both treatments (main effect for location; $P < 0.01$). At

Table 3 Steady-state exercise values at 50% of knee extension endurance time

Values are means ± S.D. *$P < 0.05$ compared with the placebo in the same environment. †$P < 0.05$ compared with the same treatment at sea level. Main effect indicates pooled difference for location (simulated altitude compared with sea level; $P < 0.05$).

	Sea level		Simulated altitude	
Measurement	Placebo	Acetazolamide	Placebo	Acetazolamide
Heart rate (beats/min)	109 ± 17	108 ± 10	113 ± 10	117 ± 15
Rating of perceived exertion	16.3 ± 2	15.2 ± 2	14.7 ± 2	16.0 ± 2
$\dot{V}_E$ (l/min)	25.9 ± 5	29.5 ± 5	35.8 ± 7†	42.2 ± 10*†
Main effect for location				
$\dot{V}O_2$ (ml/min)	759 ± 172	708 ± 98	772 ± 147	787 ± 145
$\dot{V}CO_2$ (l/min)	778 ± 71	698 ± 48	826 ± 61	851 ± 64
$\dot{V}_E/\dot{V}O_2$ (l/min)	34 ± 2	42 ± 5*	47 ± 5†	52 ± 5*†
Main effect for location				
$\dot{V}_E/\dot{V}CO_2$ (l/min)	34 ± 2	42 ± 5*	43 ± 5†	50 ± 5*†
Main effect for location				
Sao_2 (%)	97.7 ± 1	97.5 ± 1	86.3 ± 2†	88.5 ± 2*†
Main effect for location				

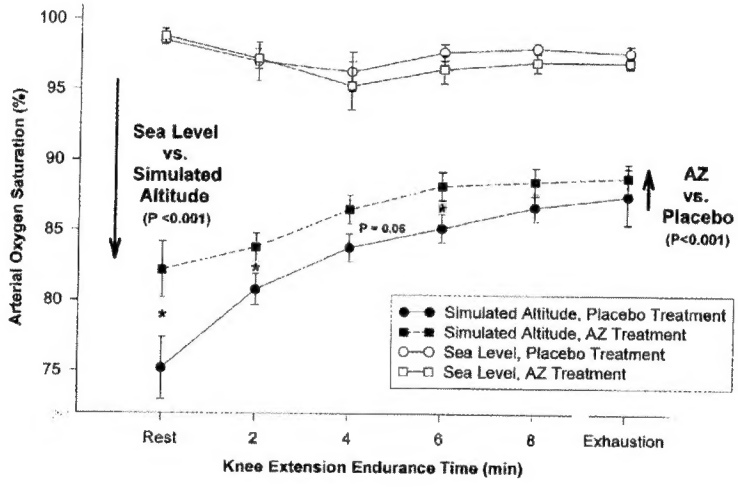

Figure 2 Sao_2 at sea level and simulated altitude with placebo or acetazolamide treatment during rest, the first 8 min of knee extension exercise and exhaustion

Overall, Sao_2 was lower at simulated altitude than sea level (main effect for location; $P < 0.001$). At sea level, Sao_2 did not differ between placebo and acetazolamide treatments and there was no difference among either treatment over time. In contrast, at simulated altitude, Sao_2 during acetazolamide treatment was higher than placebo during rest and exercise (main effect for location; $P < 0.001$) and at the indicated time points ($P < 0.05$). In addition, Sao_2 was higher during exercise than rest for each treatment.

simulated altitude, Sao_2 was significantly (main effect for location; $P < 0.01$) higher during acetazolamide treatment compared with placebo.

Figures 2 and 3 show Sao_2 and $\dot{V}E/\dot{V}CO_2$ respectively, at sea level and simulated altitude during placebo and acetazolamide treatment at rest, during the first 8 min of exercise and at exhaustion. $\dot{V}E/\dot{V}O_2$ was significantly higher during acetazolamide treatment at sea level and simulated altitude (main effect for location; $P < 0.01$). Sao_2 and $\dot{V}E/\dot{V}CO_2$ were also significantly ($P < 0.001$) higher at simulated altitude compared with sea level for both the placebo and acetazolamide treatments. Sao_2 was similar during rest and exercise at sea level. In contrast, Sao_2 increased slowly from rest during exercise at simulated altitude with significantly ($P < 0.001$) higher values during acetazolamide treatment compared with placebo.

DISCUSSION

Acetazolamide causes incomplete hydration of carbon dioxide in cells, increased renal excretion of bicarbonate, water, sodium and potassium, and increased arterial and venous blood H^+ concentrations [2–4,22]. Although

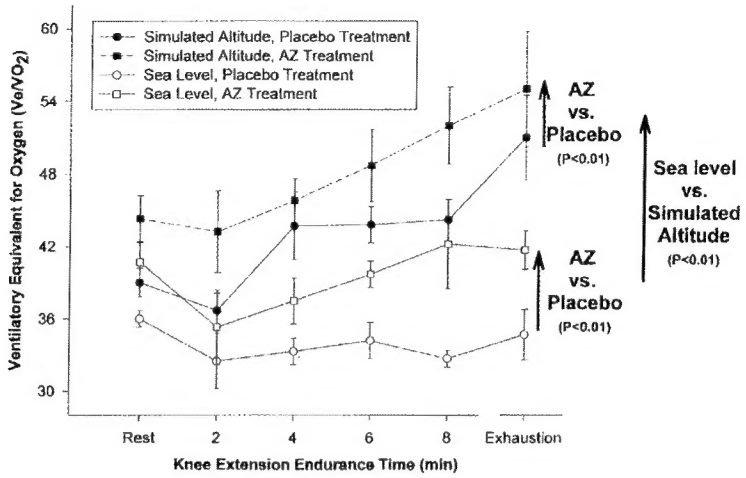

Figure 3 $\dot{V}_E/\dot{V}_{O_2}$ **at sea level and simulated altitude with placebo or acetazolamide treatment during rest, the first 8 min of knee extension exercise and at exhaustion**

Overall, $\dot{V}_E/\dot{V}_{O_2}$ was higher at simulated altitude than at sea level (main effect for location; $P < 0.01$), and was higher during acetazolamide treatment than placebo treatment in each environment (main effect for location; $P < 0.01$).

acetazolamide has been useful in the prophylaxis of AMS [1], the metabolic acidosis induced can impair ability to buffer increases in organic acids during exercise [13,14] and may adversely affect metabolic processes involved in muscular contraction [23]. Although it is commonly reported that endurance exercise performance is impaired as a result of induced acidosis or acetazolamide treatment at sea level [9,11–13,15,23], findings of impaired exercise performance during acetazolamide treatment at altitude are reported in some [7,9,12,24], but not all [3,6,11], studies.

Conflicting findings may relate to inconsistencies in experimental conditions that make it difficult to reach a consensus regarding the effect of acetazolamide on exercise performance at altitude. Among studies, there have been differences in: drug dose and administration schedule between subjects; altitude elevation, duration, ascent rate and degree of altitude acclimatization; variability and intensity of the exercise mode; recovery times between consecutive exercise bouts; diet; and degree of altitude sickness during testing [6,7,9,11,12,25,26]. A reduced hydration status due to acetazolamide treatment [27] and a central circulatory and pulmonary diffusion limitation associated with intense whole-body exercise at altitude may also have contributed to uncertainty regarding the effect of acetazolamide on exercise performance [7,16].

The experimental design of the present study attempted to minimize as many of the above potentially confounding factors as possible to determine the effect of acetazolamide on exercise performance at sea level and simulated altitude. To that end, all subjects performed exercise at identical power outputs under all experimental conditions using an exercise model that provides highly reproducible results during isolated muscle exercise performance [17,18]. Using this exercise model, instead of universally employed conventional ergometry [7,9,11,12,24], eliminated the possibility that central circulatory and pulmonary diffusion limitations, common during intense whole-body exercise, would restrict muscle perfusion and oxygen delivery to active muscle at sea level or altitude [16,20,28]. The implication is that potentially adverse effects on local muscle performance resulting from induced acidosis could probably be separated from those due to central circulatory and pulmonary diffusion limitations associated with whole-body exercise, hypoxia or both. In addition, unlike exertion during conventional ergometry, exertion during isolated muscle exercise does not exaggerate the hypoxic stress associated with altitude exposure (as evidenced by an increase in Sa_{O_2} from resting values during exercise [20]). By not exaggerating the hypoxic stress further and thereby not masking potential beneficial secondary responses resulting from the induced acidosis (e.g. increased ventilation), the effect of acetazolamide on muscle endurance performance itself at altitude could be more clearly assessed.

In the present study, the magnitude of induced acidosis during acetazolamide treatment at rest was similar for sea level and simulated altitude, and was similar to values reported previously for a comparable drug dose [12,20,24,27]. Yet, despite a similar acidosis at sea level and simulated altitude in the same subjects, our results unequivocally show that acetazolamide impaired muscle endurance performance at sea level, but not at simulated altitude. One possibility for the lack of difference in endurance performance at simulated altitude during acetazolamide treatment compared with placebo may

relate to a rapid resolution of intramuscular acidosis by non-exercising muscle [29]. In previous studies involving severe short-duration exercise (e.g. 'all-out' 30-s isokinetic cycling), there was a rapid accumulation of lactate and an associated severe intramuscular acidosis [29,30]. The related large blood lactate and various ionic concentration changes immediately after exercise cessation were subsequently buffered by non-exercised muscle [29,31]. These results indicated that intramuscular acid–base disturbances in exercising muscle resulting from severe short-duration exercise could be largely modulated by non-exercising muscle.

The implication for the present study is that rapid resolution of the acetazolamide-induced intramuscular acidosis during less severe and longer duration steady-state exercise may have prevented a further decline in endurance performance compared with placebo at simulated altitude. However, for this possibility to be tenable would require that endurance performance at sea level and simulated altitude be similarly affected during acetazolamide treatment relative to placebo. However, for the same subjects, endurance performance was adversely affected only at sea level and not simulated altitude. Moreover, this performance occurred inconsistently despite a similar level of drug-induced acidosis that resulted in similar physiological responses compared with placebo in each environment (e.g. absolute increase in $\dot{V}E/\dot{V}CO_2$). Collectively, these findings indicate that other physiological processes became effective or more effective at simulated altitude than at sea level to successfully counteract the adverse effect of induced acidosis on isolated muscle during steady-state endurance exercise. Our data suggest greater ventilation and a resulting enhanced oxygenation during acetazolamide treatment while exercising at simulated altitude.

Exercise ventilation (expressed as l/min, $\dot{V}E/\dot{V}O_2$ or $\dot{V}E/\dot{V}CO_2$) was higher at simulated altitude compared with sea level for both the placebo and acetazolamide treatments. An increase in ventilation during exercise at the same power output at altitude compared with sea level during placebo treatment is mediated by peripheral chemoreceptors sensing both an altitude-induced reduction in PaO_2 and exercise-induced increase in blood $[H^+]$ [11,32]. A further increase in exercise ventilation during treatment with acetazolamide compared with placebo at simulated altitude was probably mediated by the additional combined effects of acetazolamide-induced increases of blood $[H^+]$ and brain cell accumulation of carbon dioxide (resulting in a higher $[H^+]$) that stimulated peripheral and central chemoreceptors further [3,4,33,34]. Some [7,11,25], but not all [12,35], previous reports agree with our finding of an increase in exercise ventilation resulting from acetazolamide treatment at altitude.

The higher exercise SaO_2 at simulated altitude during treatment with acetazolamide compared with placebo was probably a consequence of the acetazolamide-induced increase in exercise ventilation [11]. Results of most previous altitude studies in which both ventilation and SaO_2 were determined during exercise indicate that, if ventilation was significantly higher during acetazolamide treatment, then SaO_2 or PaO_2 was also higher [6,11,24]. Moreover, in another study [12], acetazolamide treatment during exercise did not cause ventilation to increase and SaO_2 also did not increase. Collectively, results from previous studies agree with the results of the present study and are consistent with our hypothesis of a direct link between an acetazolamide-induced increase in ventilation and SaO_2. It is unclear why similar acetazolamide treatment at altitude increases exercise ventilation and SaO_2 in some but not all studies.

At altitude, enhanced oxygenation during acetazolamide treatment (consistent with our finding of higher SaO_2) would provide a better PO_2 gradient from capillary to exercising muscle. In addition, an acidosis-induced rightward shift of the haemoglobin dissociation curve would increase oxygen unloading from capillaries to muscle. These secondary beneficial changes at simulated altitude apparently were enough to offset the direct adverse effect on induced acidosis in exercising muscle. In contrast, at sea level, arterial oxygenation was already near maximal levels during placebo treatment and, therefore, could not physiologically change as a result of the acetazolamide-induced increase in ventilation. Since small muscle exercise is also associated with much lower peak cardiac output and minimal impact on pulmonary transit time compared with whole-body exercise [20], any acidosis-impaired oxygen loading at the lung would probably be inconsequential with regard to small-muscle performance. Overall, this interpretation is consistent with our finding in the same subjects of a decrease in performance at sea level, but no change in performance at simulated altitude despite an increase in $\dot{V}E/\dot{V}O_2$ in both environments.

In summary, acetazolamide treatment at sea level and simulated altitude caused a similar acidosis and ventilatory increase during exercise. As a result of the increase in ventilation, SaO_2 increased, capillary to muscle PO_2 gradient probably improved and performance was not impaired at simulated altitude, even though it was impaired at sea level in the same subjects. Thus, during isolated muscle exercise at simulated altitude, partial carbonic anhydrase inhibition via acetazolamide apparently can be overcome by the resulting acidosis-induced increase in ventilation that leads to a better pressure gradient for oxygen delivery to active muscles.

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